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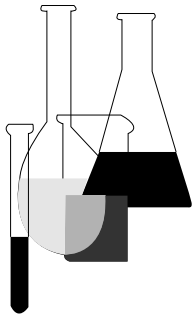
Fate, Transport and Transformation Test Guidelines

OPPTS 835.3210
Modified SCAS Test



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

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OPPTS 835.3210 Modified SCAS test.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are the OPPT guideline under 40 CFR 796.3340 Ready Biodegradability: Modified OECD Screening Test and OECD guideline 302 A Inherent Biodegradability: Modified SCAS Test.

(b) **Introductory information—**(1) **Prerequisites.** (i) Water solubility.

(ii) The organic carbon content of the test material must be established.

(2) **Guidance information.** (i) Information on the relative proportions of the major components of the test material will be useful in interpreting the results obtained, particularly in those cases where the result lies close to the ‘‘pass level.’’

(ii) Information on the toxicity of the chemical may be useful to the interpretation of low results and in the selection of appropriate test concentrations.

(3) **Qualifying statements.** (i) The method is only applicable to those organic test materials which, at the concentration used in the test:

(A) Are soluble in water (at least 20 mg DOC/L (DOC = dissolved organic carbon)).

(B) Have negligible vapor pressure.

(C) Are not inhibitory to bacteria.

(D) Do not significantly adsorb on glass surfaces.

(E) Are not lost by foaming from the test solution.

(ii) This test has been found suitable by the OECD Expert Group Degradation/Accumulation for determining the inherent biodegradability of organic chemicals under aerobic conditions.

(4) **Recommendations.** Test chemicals giving a result of greater than 20 percent loss of DOC in this test may be regarded as inherently biodegradable, whereas a result of greater than 70 percent loss of DOC is evidence of ultimate biodegradability. The use of a compound specific analytical technique on ^{14}C-labeled test substance may allow greater sensitivity. In these last cases a lower level may be regarded as evidence of inherent biodegradability.

(5) **Standard documents.** This test guideline has been based on the paper cited under paragraph (e)(1) of this guideline.

(c) **Method**—(1) **Introduction, purpose, scope relevance, application and limits of test.** (i) The method is an adaptation of the Soap and Detergent Association semicontinuous activated sludge (SCAS) procedure for assessing the primary biodegradation of alkyl benzene sulfonate. The method involves exposure of the chemical to relatively high concentrations of microorganisms over a long time period (possibly several months). The viability of the microorganisms is maintained over this period by daily addition of a settled sewage feed.

(ii) Because of the long detention period (36 h) and the intermittent addition of nutrients the test does not simulate those conditions experienced in a sewage treatment plant. The results obtained with the test substance indicate that it has a high biodegradation potential, and for this reason it is most useful as a test of inherent biodegradability.

(iii) Since the conditions provided by the test are highly favorable to the selection and/or adaptation of microorganisms capable of degrading the test compound, the procedure may also be used to produce acclimatized inocula for use in other tests. The test is applicable to water soluble, nonvolatile, organic chemicals that are not inhibitory to bacteria at the test concentration.

(2) **Reference substances.** In some cases when investigating a new substance reference substances may be useful; however, specific reference substances cannot yet be recommended. Data on several compounds used in ring tests are provided (see Table 1 under paragraph (d)(1)(ii) of this guideline) primarily so that calibration of the method may be performed from time to time and to permit comparison of results when another method is employed.

(3) **Principle of the test method.** (i) Activated sludge from a sewage treatment plant is placed in an aeration (SCAS) unit. The test compound and settled domestic sewage are added, and the mixture is aerated for 23 h. The aeration is then stopped, the sludge allowed to settle and the supernatant liquor is removed. The sludge remaining in the aeration chamber is then mixed with a further aliquot of test compound and sewage and the cycle is repeated.

(ii) Biodegradation is established by determination of the DOC content of the supernatant liquor. This value is compared with that found for the liquor obtained from a control tube dosed with settled sewage only.

(4) **Quality criteria**—(i) **Reproducibility.** The reproducibility of this modification of the method based on removal of DOC has not yet been established. When primary biodegradation is considered, very precise data is obtained for materials that are extensively degraded. The results reported

in paragraph (e)(1) of this guideline suggest 95 percent confidence limits of less than ±3 percent, and this includes interlaboratory tests. As would be expected, wider confidence limits are obtained for less biodegradable materials.

(ii) **Sensitivity.** The sensitivity of the method largely depends on the precision of the determination of DOC and the level of test compound in the liquor at the start of each cycle. At the end of the aeration period about 10 mg/L of DOC remain in the supernatant liquor of the control experiment. Assuming that the DOC determination is within ±5 percent and a level of 20 mg/L of carbon as test material is added at the start of the aearation period, then the assessment of the extent of biodegradation should be within ±6 percent for the range 80–100 percent biodegradation.

(iii) **Specificity.** The method is applicable to any nonvolatile, water-soluble, organic compound.

(iv) **Possibility of standardization.** Since the method uses a feed of real settled sewage, absolute standardization is not possible unless this feed were replaced by an artificial one. However, since the method is designed to give an indication of the biodegradability potential of a chemical and is not a simulation test, such standardization is unnecessary.

(v) **Possibility of automation.** Automation of this method would be possible but would be expensive. As the method is not labor intensive, the exercise would offer few advantages.

(5) **Description of the test procedure—**(i) **Preparations.** (A) The aeration units are cleaned and fixed in a suitable support. The air inlet tubes are connected to the supply manifold. A small laboratory scale air compressor is used to aerate the units, and the air is presaturated with water to reduce evaporation losses from the units.

(B) A sample of mixed liquor from an activated sludge plant treating predominantly domestic sewage is obtained. Approximately 150 mL of the mixed liquor are required for each aeration unit.

(C) The organic carbon analyzer is calibrated using potassium hydrogen phthalate.

(D) Stock solutions of the test compounds are prepared: the concentration normally required is 400 mg/L as organic carbon which gives a test compound concentration of 20 mg/L carbon at the start of each aeration cycle if no biogradegradation is occurring.

(E) The organic carbon content of the stock solutions is measured.

(ii) **Test conditions.** A high concentration of aerobic microorganisms is used, and the effective detention period is 36 h. The carbonaceous material in the sewage feed is oxidized extensively within 8 h of the start of

each aeration cycle. Thereafter, the sludge respires endogeneously for the remainder of the aeration period, during which time the only available substrate is the test compound unless this is also readily metabolized. These features, combined with daily reinoculation of the test when domestic sewage is used as the medium, provide highly favorable conditions for both acclimatization and biodegradation.

(iii) **Performance of the test.** (A) A sample of mixed liquor from a suitable activated sludge plant is obtained and aerated during transportation to the laboratory. Each aeration unit is filled with 150 mL of mixed liquor and the aeration is started. After 23 h, aeration is stopped, and the sludge is allowed to settle for 45 min. The tap is opened and 100 mL of the supernatant liquor withdrawn. A sample of settled domestic sewage is obtained immediately before use, and 100 mL are added to the sludge remaining in each aeration unit. Aeration is started anew. At this stage no test materials are added, and the units are fed daily with domestic sewage only until a clear supernatant liquor is obtained on settling. This usually takes up to 2 weeks, by which time the DOC in the supernatant liquor at the end of each aeration cycle should be less than 12 mg/L.

(B) At the end of this period the individual settled sludges are mixed, and 50 mL of the resulting composite sludge are added to each unit.

(C) Settled sewage (100 mL) is added to the control units and 95 mL plus 5 mL of the appropriate test compound stock solution (400 mg/L) to the test units. Aeration is started again and continued for 23 h. The sludge is then allowed to settle for 45 min and the supernatant drawn off and analyzed for DOC.

(D) The fill and draw procedure under paragraph (c)(5)(iii)(A) of this guideline is repeated daily throughout the test.

(E) Before settling it may be necessary to clean the walls of the units to prevent the accumulation of solids above the level of the liquid. A separate scraper or brush is used for each unit to prevent cross contamination.

(F) The DOC in the supernatant liquors is determined daily, although less frequent analysis is permissible. Before analysis the liquors are filtered through washed 0.45 μm membrane filters and certifuged. Temperature of the sample must not exceed 40 °C while it is in the centrifuge.

(G) The length of the test for compounds, showing little or no biodegradation is indeterminate, but experience suggests that this should be at least 12 weeks.

(d) **Data and reporting—**(1) **Treatment of the results.** (i) The results of analysis for DOC in the supernatant liquors of the test units and the control units are plotted against time. As biodegradation is achieved the level found in the test will approach that found in the control. Once

the difference between the two levels is found to be constant over three consecutive measurements, three further measurements are made and the percentage biodegradation of the test compound is calculated by the following equation:

$$\text{Percent biodegradation} = 100\,[O_T - (O_t - O_c)]/O_T$$

where

O_T = concentration of test compound as organic carbon added to the settled sewage at the start of the aeration period.

O_t = concentration of DOC found in the supernatant liquor of the test at the end of the aeration period.

O_c = concentration of DOC found in the supernatant liquor of the control.

(ii) The level of biodegradation is therefore the percentage elimination of organic carbon, under the following Table 1:

Table 1—Examples of Results of SCAS Test on Various Compounds Used in the OECD/EEC Ring Test

Test compound	O_T (mg/L)	$O_t - O_c$(mg/L)	Percentage biodegradation/bioelimination
4-Acetylaminobenzene sulfonate	17.2	2.0	85.0
Tetrapropylenebenzene sulfonate	17.3	8.4	51.4
4-Nitrophenol	16.9	0.8	95.3
Diethylene glycol	16.5	0.2	98.8
Aniline	16.9	1.7	95.9

Duration of test 40 days.

Results Found for Cyclopentane Tetracarboxylate

O_T (mg/L)	$(O_t - O_c)$ (mg/L)	Percentage biodegradation/ bioelimination
17.9	3.2	81.1

Duration of test 120 days.

(iii) If from the outset there is no difference between the control and the test, or the difference between the two remains constant at a level less than would be expected if no degradation had taken place, further tests are necessary to distinguish between biodegradation and adsorption.

(e) **References.** The following references should be consulted for additional background information on this test guideline.

(1) A Procedure and Standards for the Determination of the Biodegradability of Alkyl Benzene Sulfonate and Linear Alkylate Sulfonate. *Journal of the American Oil Chemists Society* 42:986-993 (1965).

(2) [Reserved]